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An LC–MS/MS method for determination of jujuboside A in rat plasma and its application to pharmacokinetic studies

Changhui Liu, Yingyi Li, Yanhua Zhong, Xiaotao Huang, Xia Zheng, Neng Li, Shaoling He, Suiqing Mi, Ningsheng Wang*

Institute of Clinical Pharmacology, Guangzhou University of Chinese Medicine, No. 12, Jichang Road, Guangzhou 510405, Guangdong Province, China

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ABSTRACT

A novel, simple, and sensitive method for the determination of jujuboside A in rat plasma using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was developed. Following solid-phase extraction, measurement of jujuboside A was performed by negative ion electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. The limit of detection was 1.25 ng/mL, and the lower limit of quantification was 5 ng/mL in rat plasma. Good linearity was obtained over the range of 6.25–500 ng/mL, and the correlation coefficient was better than 0.998. The intra- and inter-day precisions ranged 4.4–7.5% and 2.9–10.7%, respectively. The accuracy derived from QC samples ranged 3.2–7.8% and 2.2–3.5%, respectively. The recovery ranged from 72.9 to 75.1% and the matrix effect from 96.7 to 105.3%. The analyte was stable under various conditions (at room temperature, during freeze–thaw, in the autosampler and under deep-freeze conditions). The developed method was successfully applied to the pharmacokinetic study in rats.

1. Introduction

Semen Ziziphi Spinosae (suanzaoren in China), the mature seed of *Ziziphus jujube* Mill *var spinosa* (Bunge) Hu ex H F Chou (Rhamnaceae), has been used efficiently and widely as a sedative agent to treat insomnia and anxiety symptoms for thousands of years in China and Japan [1,2]. Several ingredients from suanzaoren containing saponins, phenolic acid, spinosins and flavonoids have been found to have hypnotic-sedative and anxiolytic effects [3,4]. Jujuboside A (JuA, Fig. 1), the triterpenoid saponins isolated from suanzaoren, was proved to be a major active component of suanzaoren [5–7]. It is shown that JuA is a non-competitive inhibitor of CaM [7] and has an inhibitory effect on the locomotor activities of mice [8]. JuA also has an inhibitory effect on the rat hippocampal formation in vivo and in vitro and decreases the slopes of excitatory postsynaptic potential through glutamate-mediated excitatory signal pathway [9,10]. Recently, JuA is found to modulate the γ-amino-butyric acid ($GABA_A$) receptor subunit gene expression of hippocampal neurons in different terms in vitro [11]. However, even with the comprehensive research on bioactivity, there was little information about its pharmacokinetic profile. Therefore, it is necessary to study and describe the pharmacokinetic properties of juA.

Several analytical methods for the quantification of JuA have been reported, such as high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) [12,13] and ultraviolet detection (HPLC-UV) [14], capillary electrophoresis (CE) [15,16] and high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) [5]. Nevertheless, due to the lack of analytical sensitivity and low selectivity, HPLC-ELSD and UV methods mainly focus on the quantification of JuA in raw materials or pharmaceutical preparations and are not suitable for the determination of JuA in biological fluids. However, information on determination of JuA on bio-samples or pharmacokinetic study of JuA in experimental animals is unavailable until now. For further clinical application of suanzaoren it is necessary to develop one sensitive method for the determination of JuA in bio-samples and to obtain pharmacokinetic information of JuA in experimental animals. Therefore, in the present study, we have established and validated a sensitive LC–MS/MS method for the quantification of JuA and successfully applied the newly developed method to the pharmacokinetic study of JuA in rats after oral administration.

2. Experimental

2.1. Chemicals and reagents

JuA (Fig. 1) and the internal standard, astragaloside IV (ASIV, Fig. 1), both with the purity of >98.5% were provided by the

* Corresponding author. Tel.: +86 20 36585534; fax: +86 20 36588015.
E-mail addresses: nsw@adr.com.cn, stephenchliu@hotmail.com (N. Wang).

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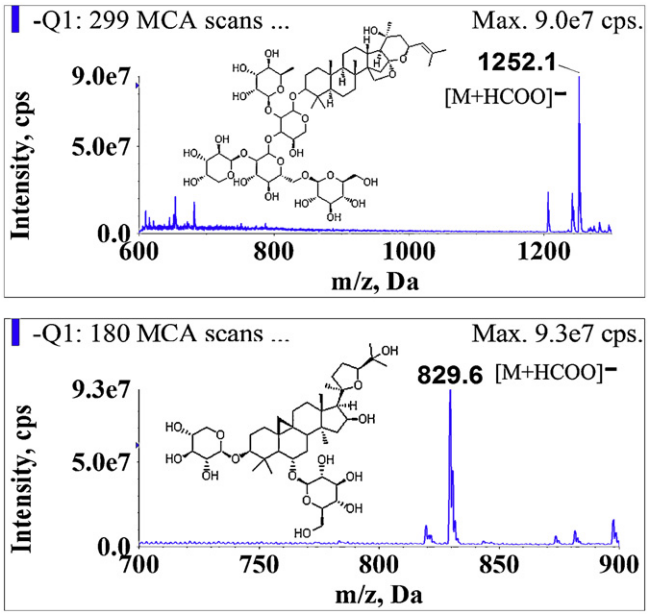

Fig. 1. Chemical structures and negative ion electrospray mass scan spectra of JuA (A) ($C_{58}H_{94}O_{26}$, MW = 1207.3) and ASIV (B) ($C_{41}H_{68}O_{14}$, MW = 784.9).

National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Ammonium formate and formic acid were purchased from Sigma (St. Louis, MO, USA). The water used was purified on a Milli-Q water purification system (Millipore, Milford, MA, USA). Solid phase extraction cartridges (Phenomenex Strata-X cartridge, 3 mL, and 60 mg) were purchased from the Phenomenex Corporation (USA).

2.2. LC–MS/MS instrument and conditions

The LC–MS/MS system was made up of an API 4000 mass spectrometer (Applied Bio-systems, MDS Sciex Toronto, Canada) equipped with an electrospray ionization (ESI) source system, and an Agilent 1200 HPLC system (Wilmington, DE) consisting of a vacuum degasser, a binary pump and an auto-sampler. JuA and ASIV (IS) were separated on a Phenomenex Gemini C_{18} column (50 mm × 2.0 mm, 5 μm, Phenomenex Inc.) equipped with a Phenomenex guard column, eluting with a gradient mobile phase system, which consisted of methanol–5 mmol/L ammonium formate buffer (0 → 1.1 min 25:75, 1.1 → 2.1 min 25:75–90:10 and maintaining for 5 min, 7.1 → 8.1 min 90:10–25:75 and maintaining for 6 min for initial equilibrium) containing 0.1% (v/v) formic acid as a modifier at a flow-rate of 0.25 mL/min. The sample injection volume was 5 μL and the column temperature was maintained at 40 °C. The ion spray voltage was set at −4500 V. The instrument parameters, viz., nebulizer gas, curtain gas, auxiliary gas and collision gas were set at 10, 35, 30 and 30 psi, respectively. Compounds parameters, viz., declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) were −64, −20, −10, −15 V and −66, −25, −10, −15 V for JuA and ASIV, respectively. Zero air was used as the source gas while nitrogen was used as both curtain and collision gas. The mass spectrometer was operated in an ESI negative ion mode and detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of m/z 1252.3 → 1252.3 $[M+HCOO]^-$ for JuA and m/z 829.9 → 829.9 $[M+HCOO]^-$ for ASIV. Data acquisition and quantitation were performed using analyst software version 1.4.1 (Applied Bio-systems, MDS Sciex Toronto, and Canada).

2.3. Preparation of calibration standards and quality control samples

Stock solutions of JuA and ASIV (IS) were prepared both in methanol at a concentration of 0.1 mg/mL and stored at 4 °C. A series of working standard solutions of JuA ranging from 62.5 to 5000 ng/mL and an IS solution at 3000 ng/mL were prepared by diluting their stock solutions with the mobile phase. All the solutions were kept at 4 °C and were brought to room temperature before use. The plasma calibration standards of JuA were prepared as follows: 10 μL of the working solution was evaporated to dryness by a gentle stream of nitrogen, and then 100 μL of blank rat plasma was added to obtain the concentrations of 6.25, 12.5, 25, 50, 100, 250 and 500 ng/mL. Quality control (QC) samples were prepared in the same way as the calibration samples, representing low, middle and high concentrations of JuA in plasma at 10, 100 and 400 ng/mL, respectively.

2.4. Sample preparation

A simple solid-phase extraction (SPE) method was followed for the extraction of JuA from rat plasma. Rat plasma samples (100 μL) were pipetted into the 1.5-mL Eppendorf tubes, and then followed by 10 μL of the ASIV working solution (3000 ng/mL). The mixture was mixed for 30 s by a vortex before each sample was centrifuged (15,000 × g) for 5 min and then loaded onto a Phenomenex Strata-X cartridge (3 mL, 60 mg), which was preconditioned with 1 mL of methanol and then 1 mL of ultrapure water. After washing with 1 mL of water followed by vacuum drying, the analytes were then eluted with 1 mL of methanol. Subsequently, the collected eluent was completely evaporated to dryness by a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 100 μL of the mobile phase, and 5 μL of the sample solution was injected into the LC–MS/MS system for assay.

2.5. Method validation

A full method validation was performed according to the Food and Drug Administration (FDA) Bio-analytical method validation by evaluating selectivity, linearity, lower limit of quantitation (LLOQ), intra- and inter-day precisions and accuracy, recovery, matrix effect, and stability [17].

2.5.1. Selectivity

To investigate whether or not endogenous constituents interfered with the assay, six different blank rat plasma samples were assessed on the potential interferences at the LC peak region for the analyte and IS using the proposed extraction procedure and LC–MS/MS conditions.

2.5.2. Linearity

The calibration curve was acquired by plotting the ratio of sum of peak area of JuA to that of IS against the nominal concentration of calibration standards. The final concentrations of calibration standards obtained for plotting the calibration curve were 6.25, 12.5, 25, 50, 100, 250 and 500 ng/mL. The standard curve was fitted to linear regression ($y = ax + b$) using $1/x$ as the weighting factor. Blank plasma samples were analyzed to confirm the absence of interferences but were not used to construct the calibration function.

2.5.3. Limit of detection and lower limit of quantification

The lower limit of detection (LOD) of the MS analysis was defined as the analyte concentration in the plasma after the sample cleanup method that corresponds to three times the baseline noise (S/N ≥ 3). The lower limit of quantification (LLOQ) of the assay was assessed as the lowest concentration on the calibration curve that could be

quantitatively determined with an acceptable precision less than 20% and an accuracy within ±20%, which was established based on five replicates independent of the QC samples.

2.5.4. Precision and accuracy

The precision and accuracy were evaluated at concentrations of 10, 100 and 400 ng/mL. For the evaluation of intra-day precision and accuracy, five aliquots of each sample were analyzed on the same day. For inter-day precision and accuracy, five aliquots of each sample were analyzed on five consecutive days. The criteria for acceptability of the data included accuracy within ±15% standard deviation (SD) from the nominal values and a precision within ±15% RSD, except for LLOQ, of which neither accuracy nor precision should exceed ±20%.

2.5.5. Extraction recovery and matrix effect

The extraction recovery was determined by dividing the peak areas of JuA added into blank plasma and extracted using a SPE procedure with those obtained from the compound spiked into an equivalent volume of post-extraction supernatant. This procedure was repeated for five replicates at three QC concentration levels of 10, 100 and 400 ng/mL.

The matrix effect was measured by comparing the peak response of sample spiked post-extraction (A) with that of pure standard solution containing an equivalent amount of the compound (B). The ratio ($A/B \times 100$)% was used to evaluate the matrix effect. The extraction recovery and matrix effect of IS were also evaluated using the same procedure.

2.5.6. Stability

The stability of JuA in rat plasma was estimated from QC samples at three concentrations mentioned previously, using five replicates for each concentration. The stability experiments of JuA included: (a) the stability of JuA in plasma during the sample preparation was assessed by detecting samples after storage for 4 h at room temperature; (b) for freeze/thaw stability, the plasma samples were determined through three freeze (−70 °C)–thaw (room temperature) cycles and were frozen for at least 12 h at −70 °C; (c) to evaluate the stability of the treated plasma samples in the autosampler, QC samples were prepared and placed in the autosampler for a period of 24 h, and then injected for analysis; (d) the long-term stability was performed by assaying the plasma samples after storage at −70 °C for 30 days.

2.6. Pharmacokinetics study

Twelve Sprague–Dawley rats (280–320 g, six males and six females, purchased from Guangdong Experimental Animal Center, Guangzhou, China) were and maintained on a 12 h light–dark cycle with free access to food and water for five days. The rats were fasted for 12 h and had free access to water before dosing. On the day before the pharmacokinetic study, a polyethylene tube (i.d. 0.58 mm, o.d. 0.965 mm, Becton Dickinson, Sparks, MD, USA) was implanted into the right jugular vein through surgery. This catheter was used for blood sampling. After oral administration of 30 mg/kg JuA to rats by gavage, serial blood samples (250 μL) were collected before dosing and at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24 and 36 h after oral dosing, and then centrifuged at 3000 × g for 10 min immediately. All plasma samples were stored at −70 °C until analysis. The plasma concentrations of JuA at different time points were expressed as mean ± SD (standard deviation, SD). The study was approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine.

Plasma samples (100 μL) were spiked with IS and processed with the same sample preparation procedure. In addition to the plasma samples, QC samples were distributed among calibrators and unknown samples in the analytical run. Non-compartmental pharmacokinetic parameter calculations were performed using the NONMEM Program version 1.1 (GloboMax Inc., Ellicott City, MD). The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from the experimental data. The elimination half-life ($t_{1/2\beta}$) was calculated as $0.693/\beta$ where β is the elimination rate constant calculated from the terminal linear portion of the log plasma concentration–time curve. The area under the plasma concentration–time curve (AUC) from time zero to the last quantifiable time point ($AUC_{0\rightarrow t}$) and from time zero to infinity ($AUC_{0\rightarrow\infty}$) were estimated using the log-linear trapezoidal rule.

3. Results and discussion

3.1. Selection of IS

It is necessary to use an IS to obtain good accuracy when a mass spectrometer is used as a detector. Astragaloside IV (ASIV, Fig. 1), was chosen as the IS, because of its similarity of chemical structure (both for saponins), chromatographic behavior, extraction efficiency and ionization with the JuA.

3.2. Mass spectrometric detection condition

Mass spectrometric detection was carried out with an API 4000 triple quadrupole instrument equipped with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source. No specific and abundant ions for these analytes were observed in either positive or negative mode when APCI was used, probably as the saponins possess high polarity and/or weak lipophilicity [18]. In addition, low abundance and unstable $[M+H]^-$ or $[M-H]^-$ ion were formed in both positive and negative modes of ESI. To further optimize the MS conditions, different modifier reagents, viz. formic acid, ammonium formate, acetic acid, ammonium acetate, sodium and potassium, were tested, respectively. When 0.1% formic acid were added, strong and stable signals of JuA and IS can be observed in the form of their $[M+HCOO]^-$ molecular ions in the negative ESI with mass to charge ratios of m/z 1252.3 and m/z 829.9, respectively (Fig. 1). Different collision energy (CE) values for JuA and IS were tested, and no specific and abundant fragment ions of $[M+HCOO]^-$ were found in the study. At the beginning study, the selected ions m/z 1252.3 and m/z 829.9 in selected ion monitoring (SIM) mode were used to quantify JuA and IS in the rat plasma samples, respectively. The higher background noise was to be found in the chromatogram and the LLOQ was greater than 25 ng/mL, indicating the use of SIM mode might not be suitable to quantify JuA and IS in the rat plasma samples. However, when we use the MRM mode to measure JuA (m/z 1252.3 → 1252.3 $[M+HCOO]^-$) and IS (m/z 829.9 → 829.9 $[M+HCOO]^-$), the sensitivity were found to enhance about 5-fold than that of the SIM mode with LLOQ of 5 ng/mL. Compounds parameters, viz., DP, CE, EP and CXP were then optimized with the values of −64, −20, −10, −15 V and −66, −25, −10, −15 V for JuA and ASIV, respectively. Therefore we selected their $[M+HCOO]^-$ molecular ions (m/z 1252.3 → 1252.3 for JuA and m/z 829.9 → 829.9 for IS) as the detective ions for the MRM detection with negative ESI.

3.3. Liquid chromatography

Initially, the resolution and the sensitivity were found no suitable for determination JuA and IS in plasma when we used the method of isocratic elution though the retention time was short in the chromatography. However, the gradient elution of the mobile phase was offered for narrowing the peaks of JuA and IS and thus enhancing the sensitivity and resolution in the chromatography. We also have investigated various solvent systems

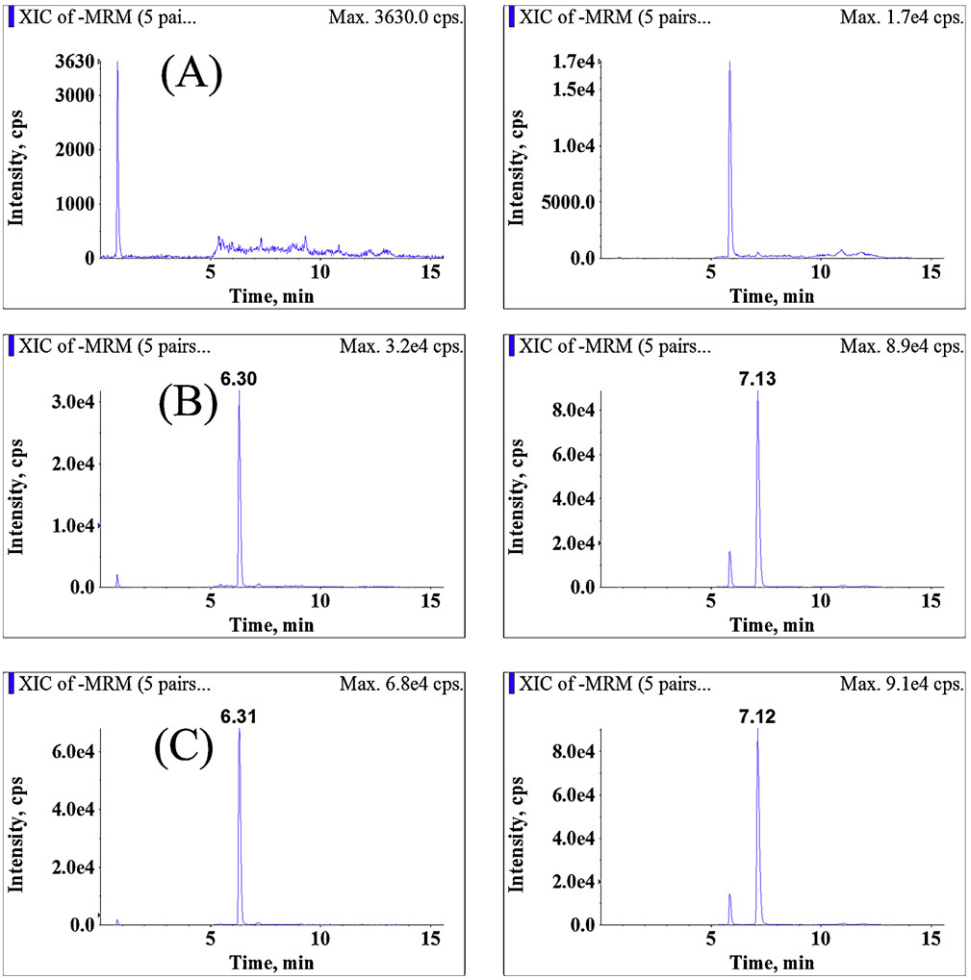

Fig. 2. Typical MRM chromatograms of JuA (left panel) and IS (right panel) in (A) rat blank plasma; (B) rat plasma spiked with 50 ng/mL of JuA and IS (C) a 1.0 h (108.3 ng/mL) plasma sample showing JuA peak obtained following oral dose of 30 mg/kg JuA to rats.

composed of mixtures of methanol, acetonitrile, using different buffers, such as formic acid, ammonium formate, and acetic acid to obtain the appropriate retention time, the best resolution and the highest sensitivity. Methanol, rather than acetonitrile, was chosen as the organic modifier because it led to lower background noise and resulted in the best resolution. In addition, the addition of 5 mM ammonium formate containing 0.1% (v/v) formic acid helped to obtain better peak shape and to enhance the ionization. Experiments were also performed with different C_{18} columns such as Phenomenex Gemini C_{18} 110A (50 mm × 2.0 mm, 5.0 μm), Alltima C_{18} (150 mm × 2.1 mm, 5.0 μm) and Dikma Spursil C_{18} (150 mm × 2.1 mm, 5.0 μm). Due to the narrow internal diameter (50 mm × 2.0 mm, 5.0 μm), Phenomenex Gemini C_{18} 110A column was finally selected for the chromatographic separation because under the current LC conditions, the column provided excellent results in terms of response, retention time and peak shapes. Therefore, the best combinations of peak shape and retention time were achieved using Phenomenex Gemini C18 110A column (50 mm × 2.0 mm, 5.0 μm) under the gradient elution with a mobile phase of methanol–5 mM ammonium formate containing 0.1% (v/v) formic acid. Typical chromatograms are shown in Fig. 2.

3.4. Sample pre-treatment

Different extracted methods of sample pre-treatment were investigated. Protein precipitation using acetonitrile or methanol gave the low extraction recovery and strong interferences from endogenous substances in plasma. Liquid–liquid extraction with various organic solvents such as ethyl acetate, n-butanol and diethyl ether and their mixtures resulted in non-reproducible recoveries and interferences in the plasma sample matrix due to the low solubility of JuA in these described above organic solvents. The extraction recovery of JuA was approximately 37% by liquid–liquid extraction using n-butanol. Hence, solid-phase extraction (SPE) was applied to extract JuA and IS from rat plasma. We found that Phenomenex Strata-X cartridge (3 mL, 60 mg) SPE column obtained satisfying extracted recovery of JuA. The average recoveries of JuA from the rat plasma were above 70% when the Phenomenex Strata-X cartridge (3 mL, 60 mg) SPE column was used.

3.5. Method validation

3.5.1. Selectivity and specificity

In the present study, the specificity and selectivity were examined using independent plasma samples from six different rats. Fig. 2 shows a typical chromatogram for the drug-free plasma (Fig. 2A), drug-free plasma spiked with JuA and IS (Fig. 2B) and an in vivo rat plasma sample after oral administration of JuA (Fig. 2C). As shown in Fig. 2, there is no significant interference from plasma found at retention times of either JuA or the IS. The retention time of JuA and the IS were approximately 6.3 and 7.1 min, respectively. The results indicated that the method exhibited good specificity

Table 1
Matrix effect of JuA in rat's plasma (mean ± SD, $n = 5$).

Spiked plasma concentration (ng/mL)	Measured concentration (ng/mL)	Matrix effect (%)	RSD (%)
10.0	10.4 ± 0.6	105.3 ± 6.3	5.8
100.0	96.6 ± 4.6	96.7 ± 4.5	4.7
400.0	392.4 ± 14.4	98.4 ± 3.2	3.3

Table 2
Extraction recovery of JuA in rat's plasma by SPE method (mean ± SD, $n = 5$).

Spiked plasma concentration (ng/mL)	Measured concentration (ng/mL)	Extraction recovery (%)	RSD (%)
10.0	7.3 ± 0.5	73.7 ± 4.9	6.8
100.0	75.1 ± 3.6	75.1 ± 3.4	4.7
400.0	291.6 ± 13.7	72.9 ± 3.7	4.6

and selectivity and was applied to plasma samples for the pharmacokinetic study.

3.5.2. Matrix effect

In this study, the matrix effect was evaluated by analyzing the low (10 ng/mL), middle (100 ng/mL) and high (400 ng/mL) QC samples. The results are summarized in Table 1. The average matrix effect values were 105.3, 96.7 and 98.4% for JuA at low, middle and high QC, respectively. The matrix effect on IS was found to be 97.1% at the tested concentration of 300 ng/mL. The matrix effect on the ionization of the analyte was not obvious under these conditions.

3.5.3. Extraction recovery

The extraction recovery was determined in five replicates by comparing the peak areas of the extracted plasma at 10, 100 and 400 ng/mL with those obtained from the direct injection of standard solutions without preparation at the same concentrations. The extraction recoveries of JuA were 73.7 ± 4.9%, 75.1 ± 3.4% and 72.9 ± 3.7% for QC samples at the concentrations of 10, 100 and 400 ng/mL, respectively. All the data are summarized in Table 2, and the extraction recovery of the IS was 74.6 ± 4.6%. The recovery of the determination of JuA and IS in rat plasma was consistent, precise and reproducible.

3.5.4. Limit of detection (LOD), the lower limit of quantification (LLOQ) and the linearity

The LOD of the JuA assays demonstrated as 1.25 ng/mL (S/N ≥ 3), and the LLOQ was 5 ng/mL. At LLOQ, the accuracy was within ±8.2%, and the precision was less than 5.7%. The calibration curves ranged from 6.25 to 500 ng/mL using seven calibration standards. The regression equation for calibration curves in plasma was $y = 0.0068x + 0.0263$ ($n = 5$), where y is the peak-area ratio [(peak area of analyte)/(peak area of IS)] versus concentration, and x is the concentration of JuA. The correlation coefficient (r^2) was ≥0.9983 for all the calibration curves, and the observed deviation was within ±6.3% for all the calibration concentrations. The method was found to be sufficiently sensitive for the determination of the pharmacokinetic analysis of JuA in rats.

3.5.5. Precision and accuracy

The precision and accuracy data for intra- and inter-day plasma samples are presented in Table 3. The assay values for both occasions (intra- and inter-day) were found to be within the accepted variable limits. The intra- and inter-day precisions ranged 4.4–7.5% and 2.9–10.7%, respectively. The accuracy derived from QC samples ranged 3.2–7.8% and 2.2–3.5%, respectively. The data indicated that the present method has a satisfactory accuracy, precision and reproducibility.

3.5.6. Stability

QC samples at three concentrations were analyzed in five replicates for studying the possible conditions to which the samples might be exposed during storage and handling. It was found that JuA was stable in rat plasma after being stored at room temperature for 4 h, after repeated three freeze–thaw cycles and after being stored at −70 °C for 30 days. In addition, the treated samples were found to be stable in the autosampler for a period of 24 h, and the results were found to be within the assay variability limits during

Table 3
Precision and accuracy for the analysis of JuA in rat's plasma ($n = 5$ days, five replicates per day).

Spiked concentration (ng/mL)	Intra-day			Inter-day		
	Measured concentration (ng/mL)	Precision (RSD, %)	Accuracy (RE, %)	Measured concentration (ng/mL)	Precision (RSD, %)	Accuracy (RE, %)
10.0	10.7 ± 0.8	7.5	7.8	10.3 ± 1.1	10.7	3.1
100.0	96.4 ± 5.7	5.9	−3.5	103.5 ± 4.3	4.1	3.5
400.0	412.7 ± 18.4	4.4	3.2	391.3 ± 11.7	2.9	−2.2

Table 4
Stability of JuA in rat plasma ($n = 5$).

Storage conditions	Concentration (ng/mL)		RSD (%)	RE (%)
	Spiked	Measured (mean ± SD)		
At room temperature for 4 h	10.0	9.6 ± 0.5	5.2	−3.9
	100.0	99.2 ± 6.6	6.7	−0.8
	400.0	408.4 ± 15.3	3.7	2.1
After three freeze/thaw cycles in plasma	10.0	10.4 ± 1.1	10.6	10.7
	100.0	93.8 ± 9.7	10.3	−6.2
	400.0	391.7 ± 10.8	2.7	2.0
In the auto-sampler for 24 h	10.0	10.1 ± 0.2	1.9	1.2
	100.0	101.7 ± 3.8	3.7	1.6
	400.0	403.9 ± 8.7	2.2	0.9
Long-term stability (at −70 °C for 30 days)	10.0	10.5 ± 1.2	11.4	11.8
	100.0	108.4 ± 6.3	5.8	8.3
	400.0	421.2 ± 22.7	5.3	5.2

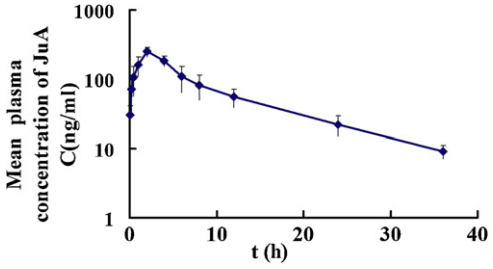

Fig. 3. Plasma concentration–time profiles of JuA in rat plasma after oral administration dose of 30 mg/kg of JuA to rats ($n = 12$).

Table 5
Main pharmacokinetic parameters of JuA after oral administration of 30 mg/kg to rats ($n = 12$, mean ± SD).

Parameters	Mean ± SD
Kel (h^{-1})	0.1022 ± 0.023
$t_{(1/2\beta)}$ (h)	6.7 ± 0.9
$AUC_{0\rightarrow 36}$ (ng/mL h)	1989.6 ± 421.7
$AUC_{0\rightarrow \infty}$ (ng/mL h)	2159.1 ± 401.4
V_d (L)	131.3 ± 38.6
Cl_{tot} (L/h)	13.95 ± 2.47
T_{max} (h)	2.0 ± 0
C_{max} (ng/mL)	252.4 ± 39.7

the entire process. All results of the stability tests are summarized in Table 4.

3.6. Pharmacokinetic study

We applied the newly developed LC–MS/MS method to the pharmacokinetic study of JuA and successfully obtained a series of the pharmacokinetic data of JuA in twelve rats after oral administration of 30 mg/kg. The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the plasma pharmacokinetics of JuA in rats. The mean plasma concentration–time profiles of JuA after oral administration is illustrated in Fig. 3, and the major pharmacokinetic parameters of JuA after oral administration were calculated by a non-compartmental model and are presented in Table 5. The values of C_{max} and T_{max} were obtained directly from experimental observations. The mean of C_{max} and T_{max} were 252.4 ± 39.7 ng/mL and 2 ± 0 h, respectively. Plasma concentration declined with at $_{(1/2\beta)}$ of 6.7 ± 0.9 h. $AUC_{0\rightarrow 36}$ and $AUC_{0\rightarrow \infty}$ values obtained were 1989.6 ± 421.7 ng h/mL and 2159.1 ± 401.4 ng h/mL, respectively. These results indicated that JuA was rapidly absorbed and eliminated slowly in rats (Fig. 3).

4. Conclusions

A novel, simple and sensitive LC–MS/MS method has been developed for quantification of JuA in rat plasma using solid-phase extraction for the first time. This method was completely validated and applied to a pharmacokinetic study of JuA in rats after oral administration at a dose of 30 mg/kg. The preliminary pharmacokinetic behavior of JuA was firstly elucidated.

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